Helium Analysis by Gas Chromatography with Thermal Conductivity Detection

1 Introduction

Asphyxiation with helium, both as a means of suicide and accidentally in practitioners of autoerotic asphyxia, is sometimes encountered in death investigations. Breathing pure helium produces almost no secondary physiological responses when compared to other common asphyxiants such as nitrogen (narcosis) and carbon monoxide (severe nausea), and compressed helium is readily available from party supply stores.

2 Scope

This procedure allows for exclusionary screening of helium that may be present in biological and non-biological samples. This document applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

Thermal conductivity detectors (TCD) function by measuring the change in temperature of a heated wire placed at the exit of a gas chromatography (GC) column that occurs when an analyte with a thermal conductivity different than that of the carrier gas exits the GC column. Normally either hydrogen or helium is used as a carrier gas with a TCD, since these gases have much higher thermal conductivity (at least 3-fold greater at 500 K) than any other gases, providing strong response. For this method, the normal practice is reversed, and nitrogen, with a moderate thermal conductivity (38 W/K at 500 K) is used as carrier gas, providing high sensitivity for helium (222 W/K at 500 K). Specificity is enhanced by the fact that relatively few gases have higher thermal conductivities than nitrogen, meaning that only a few compounds can produce interfering signals. At 500 K, the only common room-temperature gases with thermal conductivity differences greater than 5% of that for helium are: ammonia (7%), ethane (8%), ethylene (6%), hydrogen (>100%), methane (15%), and neon (17%).

4 Specimens

This procedure can be performed on a variety of biological fluids and tissue samples. Specimens for this exam should be collected as soon as possible after death and must be kept under a gas-tight seal until they are analyzed. For fluid specimens, the preferred container is a vacuum-sealed blood collection tube approximately 2/3 to 3/4 full. A 20 mL crimp-top headspace vial is

suitable for small tissue samples. For large tissue samples, the best container is a new metal paint can of the smallest size necessary to contain the sample.

5 Equipment/Materials/Reagents

a. Gas chromatograph equipped with thermal conductivity detector and J&W HP-Molesieve 30 m x 0.32 mm x 12.00 µm column (or equivalent)

b. 16 x 100 mm disposable glass culture tubes

c. deionized water

d. fold-over rubber septa

e. vacuum source

f. plastic syringe, 3 cc

g. syringe needles (various sizes)

h. syringe filters (25 mm 0.22µm PTFE)

i. centrifuge

j. heating block with thermometer

k. electrical tape (or another well-sealing adhesive tape)

l. standard GC syringe, 10 µL

m. hammer and metal probe/punch

n. volumetric flasks (100-mL)

o. basin or other container suitable for filling with water and inversion of flasks

p. routine laboratory supplies, including disposable pipettes, test tube racks, etc.

6 Standards and Controls

a. Compressed Air Supply

b. Air Standard:

An air standard serves to demonstrate the absence of helium or target analyte in the source of air used for the procedure. The air standard is made by filling a 100 mL volumetric flask completely with deionized water. The flask is then inverted in a deionized water bath and the water is displaced by air taken from the laboratory compressed air supply. The flask is then capped with a fold-over rubber septum while still inverted in the water bath. Alternatively, an air standard may be obtained by simply sampling the ambient atmosphere with a standard 10 µL GC syringe.

c. High Purity Helium (GC-grade or better)

d. High Purity Helium Standard:

The high purity helium standard is analyzed to demonstrate that the target analyte source material is free of interferences and as a source for creating the mixed helium/air control. It is prepared in the same manner as the Air Standard, substituting a high purity helium source for the laboratory compressed air supply.

e. Mixed Helium/Air Control (1% helium in air):

Prepare an air standard as in (b) above. Then use a 3 mL syringe with a fine-gauge needle to transfer 1.0 mL of the high purity helium standard into this flask. A sample of this control is analyzed prior to each batch of samples to demonstrate that the instrument is performing properly.

f. Drug-Free Blood

Obtained from Cliniqa or an equivalent approved supplier.

g. Negative Control:

Measure 9 mL of drug-free blood into a 16 x 100 mm culture tube and cap with a fold-over rubber septum. Centrifuge at low speed (≤1000 rpm) for 5 min and, using a fine-gauge needle, vent the tube headspace to the laboratory vacuum system for about 5 s. A negative control will be analyzed with every batch of samples.

h. Positive Control:

Measure 9 mL of drug-free blood into a 16 x 100 mm culture tube and cap with a fold-over rubber septum. Run a long large-gauge needle through the septum into the blood sample and use a short fine-gauge needle, with an attached syringe filter, to vent the tube headspace. Gently bubble high-purity helium through the blood sample for about 30 min. The blood sample will generate copious quantities of foam, and the vent needle must be equipped with a syringe filter to prevent the sample from foaming out of the tube. After this sparge, centrifuge the sample at low speed (≤1000 rpm) for 5 min and, using a fine-gauge needle, vent the tube headspace to the laboratory vacuum system for about 5 s. A positive control will be analyzed with every batch of samples.

7 Sampling

Representative portions of the specimens are obtained. See TOX101 for further details.

8 Procedure

a. Where possible, centrifuge samples at low speed (≤1000 rpm) for 5 min prior to equilibration and analysis. This will help prevent contamination of the sampling syringe with biological material.

b. Place all controls and unknowns into a laboratory heating block set at approximately 36°C to equilibrate the headspace. The container type for the unknown samples may preclude placement in a heating block in which case another suitable equilibration method should be substituted. Equilibrate for at least 30 minutes.

c. While the samples are equilibrating, perform the necessary QC checks for the GC-TCD instrument. The Air Standard, the High Purity Helium Standard, and the Mixed Helium/Air Control will be analyzed at this time to verify proper instrument performance.

d. Using a 10 µL standard GC syringe sample the headspace of each control or unknown and analyze using the conditions given below. For headspace vials and Vacutainer (or similar) tubes, the headspace is sampled directly through the container septum. For specimens in paint cans (or similar containers) use a hammer and a metal punch or probe to make a pinhole in the lid of the container. Immediately cover this hole with a double layer of electrical tape, and then sample the headspace through the electrical tape.

9 Instrumental Conditions

Following are the operating parameters for the instrument used in this procedure.

9.1 Gas Chromatograph Parameters

Oven Parameters		Column Parameters		Inlet and Carrier Parameters	
temperature 1	35ºC, isothermal	type	HP-Molesieve	inlet temp.	200ºC
hold 1	10 min	length	30 m	injection mode	manual, 1-10 µL
		internal diameter	0.32 mm	carrier gas	nitrogen
		film thickness	12 µm	carrier mode	constant flow
				flow	1.0 mL/min
				split ratio	2:1

9.2 Thermal Conductivity Detector

temperature	250ºC	makeup gas	nitrogen
reference flow	20 mL/min	makeup gas flow rate	5.0 mL/min
polarity	negative	reference gas	nitrogen
data sampling rate	5 Hz	reference gas flow	20.0 mL/min

10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Evaluation of results should be based upon comparison of analytical data for an unknown sample to data from analysis of positive and negative control samples.

10.1 GC-TCD Performance Criteria

The Air Standard should be free of helium or target analyte. The Helium Standard (or target standard) should be free from other interferences. The air peak and the helium (or target) peak should be well-resolved.

10.2 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.2.1 Retention Time

The retention time of the presumptive helium peak should be within ±2% of the retention time obtained from injection of a positive control sample.

10.2.2 Signal-to-Noise

To justify the existence of a peak, its signal-to-noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold-greater than that for any observed peak at similar retention time in a negative control injected just prior to the sample.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. Limit of Detection: This method will detect helium at a level of 0.5% v/v in air standards. The response for a 1% v/v standard of helium in air is less than 5% of that observed for the positive control blood specimen.

b. Interferences: None known.

c. Results of this analysis are exclusionary in nature. At present, there is no method available to confirm presumptive positive helium results. Appropriate caution should be used in reporting presumptive positive analytical results.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance. When preparing the positive control blood sample, ensure that a syringe filter is attached to the vent needle in order to prevent aerosol formation from the blood sample.

15 References

CRC Handbook of Chemistry and Physics, 89th Ed., section 6, pp. 206 – 207.

Rev #	Issue Date	History
1	01/03/2013	11.2 - Updated chromatography decision criteria
2	03/15/2021	1 - Removed interpretive language. 2 - Updated to current scope language. 4, 5 - Removed brand names where possible. 5, 6 - Moved items between sections to match current practices and renumbered as needed. 7 - Removed section (calibration) and renumbered subsequent sections. Updated sampling statement. 9 - Added standard instrument parameter preamble text. 12 - Updated language to "Measurement Uncertainty" 15 - Removed references to internal FBI Laboratory documents.

Approval Redacted - Signatures on File

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